Elucidating the Role of *DANCR* in Leukemic Stem Cells of Acute Myeloid Leukemia

Research Thesis

Presented in partial fulfillment of the requirements for graduation with *research distinction* in the undergraduate college of The Ohio State University

By:
Allison E. Walker

The Ohio State University
May 2019

Project Advisor: Dr. Adrienne Dorrance

This thesis includes data published in Nature Publishing Group: Leukemia

Bill, Marius, et al. "Expression and Functional Relevance of Long Non-Coding RNAs in Acute Myeloid Leukemia Stem Cells." *Leukemia*, 2019, doi:10.1038/s41375-019-0429-5.

Table of contents

Acknowledgments

I would like to thank my principal investigator, Dr. Adrienne Dorrance, who invited me to join our lab in the Fall of 2016. I would also like to thank the Dorrance, Garzon, and Bloomfield labs for contributing to this project and for their continued support, patience and persistent sense of humor.

I would like to thank Dr. David M. Lucas and Ms. Donna Bucci from the Leukemia Tissue Bank of The Ohio State University for sample support (CCC Support Grant: P30CA016058). This work is supported by the Leukemia Clinical Research Foundation (M.B.), Gabrielle's Angels Foundation and ASH Bridge Grant (A.M.D).

Thank you to all my professors at The Ohio State University in Columbus, Ohio and my teachers at Mayo High School in Rochester, Minnesota. You have taught me everything I know. Literally.

Finally, I would like to thank my parents. Because you are a part of the E and T more than the S in STEM, I don't expect you to understand all the words in this thesis. However, I appreciate your questions, grammatical edits, and late-night phone calls. You rock.

Abstract:

Acute Myeloid Leukemia (AML) is a malignant disease associated with poor outcomes. One of the major clinical challenges is suffering relapse after achieving a complete remission. Leukemic Stem Cells (LSC) persist in the minimal residual disease cell populations and cause AML relapse because they are assumed to be more resistant to standard chemotherapy. Therefore, these cells must be specifically targeted in AML patients. Long non-coding RNAs (lncRNA) are distinguished by having more than 200 nucleotides and regulate cellular function. Their role in LSCs is yet to be determined.

We analyzed 377 AML patients for levels of expression of various lncRNAs. Gene expression signatures (GES) from AML LSC populations have been defined and expression of a 'core enriched' (CE) GES, representing 44 genes deregulated in LSCs, is correlated with lower survival rates. We studied associations between the CE-GES and lncRNA expression profiles and identified 161 lncRNAs that were found to be deregulated in the AML patients. Of these, 152 lncRNAs were found to be higher expressed in high CE than low CE patients, indicating that they present in AML patient's gene expression profiles that have more characteristics of stem cells. Among these lncRNAs, *DANCR* was found in patients with high CE score.

To examine effects of *DANCR* in LSCs, we performed a long-term culture initiating cell (LTC-IC) assay which quantified the frequency of stem cells in a given population. Cells were treated with transferrin-conjugated anionic lipopolyplex nanoparticle (transferin-NP-siDANCR or transferin-NP-siSCR controls) which work as a delivery

method for treatment. We found significantly fewer amount of stem cells were present in the DANCR knockdown population compared to scramble controls.

Next, Real Time PCR (qRT-PCR) was performed to compare *DANCR* expression in AML patients' bone marrow to healthy bone marrow. The amount of *DANCR* expression varied between patients but was significantly elevated compared to the control bone marrow ($p<0.01$). Specifically, in LSC enriched populations (n=4), *DANCR* expression was significantly higher within individual patients (n=4). To determine the role of *DANCR* in LSC self-renewal, a colony forming unit (CFU) was performed with *DANCR* knockdown in CD34+ AML patients (n=3). Nanoparticle was used in CD34+ enriched AML cells to perform the CFU. After replating, we found significantly fewer colonies in *DANCR* knockdown cells when compared to a scramble control ($p=0.03$, average decrease: 38.9%). A celltrace violet (CTV) assay was performed to assess quiescence of primary AML blast cells after *DANCR* knockdown. A significant decrease in the number of quiescent (i.e. CTV max/CD34+) cells were found in the *DANCR* knockdown group compared to scramble controls ($p<0.05$). Finally, *in vivo* experiments were performed by conditioning mice with radiation followed by treatment of Tf-NP-anti-*DANCR*. This caused a significant decrease in engraftment of mice compared to scramble control ($p<0.001$)

Our data show that the lncRNA, *DANCR,* is overexpressed in LSCs. Our experiments suggest that *DANCR* may play a functional role in AML.

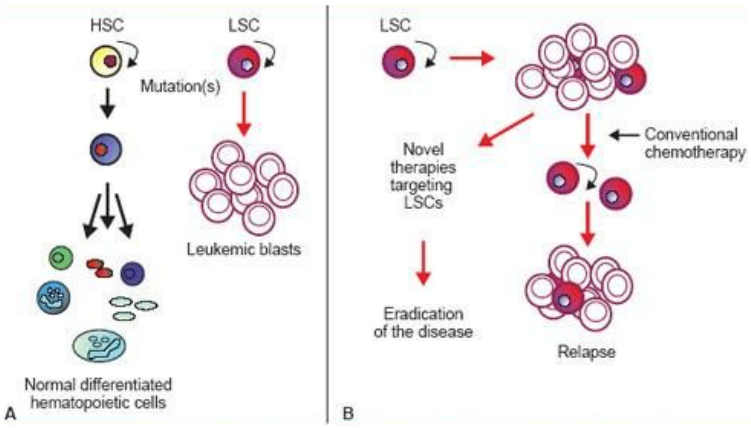
Section 1: Introductions

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a malignant disease that is highly heterogeneous with respect to genetic abnormalities and outcome. In approximately 20% of newly diagnosed patients, initial chemotherapy fails and about 65% of patients who achieve a complete remission, relapse during their lifetime. Although there have been many advances in understanding of AML, long-term survival rates are low.[1-3]

AML is characterized by immature, abnormally proliferating myeloid cells. Cell subpopulations of the disease include bulk blast cells, the majority of cells, and rare leukemic stem cells (LSC) that are relatively quiescent.

Leukemic Stem Cells



Alexey Bersenev: Cell Trials 2007

The LSC model postulates that AML consists of heterogeneous cell populations. The LSC subpopulation is essential for leukemia maintenance and therefore can be a potential pharmacological target for eradication.

A potential cause of relapse is the persistence of LSCs and their quiescent nature.[4-9] This quiescence also makes them less sensitive to standard chemotherapy, which targets mainly rapidly dividing cells.[10-12] A better understanding of the effects of LSCs might lead to the development of novel therapies that prevent relapse and improve outcome by eliminating LSCs.[6, 13-14]

Long non-coding RNAs

Long non-coding RNAs (lncRNAs) have a transcript length of 200 nucleotides or longer and are located within overlapping antisense transcripts or intergenic stretches of protein coding genes. LncRNAs make up a heterogeneous group of transcripts that regulate cellular functions necessary for cell survival including imprinting, cell cycle, epigenetic regulation and apoptosis, however, they have no protein coding potential.[15-17] One role of lncRNAs is to remodel and loop chromatin in cancer cells while also facilitating cross talk between different mechanisms.[16] The role of lncRNA is yet to be determined in LSCs.

Core Enriched Gene Expression Signature and *DANCR*

A ‘core enriched’ gene expression signature (CE-GES), created by Eppert et al, uses a formula to correlate LSC gene signatures with patient clinical outcomes.[4] GES from AML LSC populations have been defined and expression of a CE-GES, representing 44 genes deregulated in LSCs, is correlated with lower survival rates. We studied associations between the CE-GES and lncRNA expression profiles and identified 161 lncRNAs that were found to be deregulated in the AML patients. Of these, 152 lncRNAs were found to be higher expressed in high CE than low CE patients,

indicating that they present in AML patient's gene expression profiles that have more characteristics of stem cells. Among these lncRNAs, *DANCR* was found in patients with high CE score.

DANCR was also chosen among the top deregulated RNAs due its conservation between humans and mice, and its characterization as an intergenic lncRNA. These conditions are optimal for experimentation and translation of results.

Section 2: Project Description

Aims

- Identification of a lncRNA signature that is associated with LSCs.
- Elucidation of the role of the lncRNA *DANCR* in LSCs.

Project Summary

AML is a clonal, neoplastic disease that is heterogeneous at the molecular, cytogenetic, cellular and clinical level. While advances have been made towards understanding the biology and pathogenesis of AML, the prognosis for AML patients is still poor.[1] Our view of a morphologically homogeneous and functionally static blast population present in the bone marrow (BM) and blood of AML patients has now evolved into our current perspective of multiple dynamic and heterogeneous cell subpopulations. These cells include the relatively rare leukemia stem cells (LSCs). LSCs have acquired abnormal self-renewal and partial maturation ability, are considered responsible for disease initiation and maintenance and are more quiescent, making them more resistant to standard chemotherapies, leading to relapse.[4,12,15] Therefore, targeting LSCs may represent an essential step for complete eradication of the disease.

LncRNAs are 200 nucleotides or longer in intergenic stretches or overlapping gene coding sequences. They have cellular functions including gene regulation and imprinting, however, they are not translated into proteins.[16] Their role in LSCs has yet to be determined.

To identify a lncRNA specific in LSCs, we analyzed BM samples (n=377) of cytogenetically normal AML (CN-AML) for a core enriched gene expression signature.

We correlated these data with lncRNA profiles from whole transcriptome sequencing (RNA-seq). We found 161 lncRNAs to be deregulated in AML patients. One of the top upregulated lncRNAs, *DANCR,* was previously discussed in publications on HCC which was found to promote ‘stemness’ by interacting with β-catenin and the WNT pathway.[17] Our projection is to continue to define a role of *DANCR* in LSCs by determining its role in AML patients.

Section 3: Methods and Results

Core Enriched, Gene Expression Signature Analysis

To identify lncRNAs that may play a functional role in LSCs, we analyzed 365 younger (<60 years old) and 76 older (≥60 years old) adult cytogenetically normal AML (CN-AML) patients to derive a LSC-specific lncRNA signature.[4] This analysis compares gene expression signatures associated with LSCs and HSCs.[7] We studied associations between the CE-GES and lncRNA expression profiles and identified 161 lncRNAs that were found to be deregulated in the AML patients. Of these, 152 lncRNAs were found to be higher expressed in high CE than low CE patients, indicating that they present in AML patient's gene expression profiles that have more characteristics of stem cells. Among these lncRNAs, *DANCR* was found in patients with high CE score. (Figure 1). Because lncRNAs are a group of heterogeneous RNAs and are comprised of multiple different RNA classes, we further classified the lncRNAs using the GENCODE v22 database. It has been shown that the most common were long intergenic non-coding RNAs (lincRNAs; 30%), antisense RNAs (19%) and sense intronic RNAs (13%).[19] Looking at individual lncRNAs, *DANCR* was among the top deregulated lncRNAs in both, younger and older, CN-AML patients with CE^{High} score. We chose to analyze the potential regulatory role of *DANCR* further because to it was previously described to play an important role in de-differentiation of epidermal progenitor cells and malignant transformation of various tissues.[17, 20-22] Additionally, *DANCR* has been shown to regulate the WNT pathway, which is an essential for stem cell function. Finally, *DANCR* is highly conserved between humans and mice with well-defined locations.[17, 23-24]

Because *DANCR* is an intergenic lncRNA, we avoided results that may have been due to gene expression. For these reasons, we hypothesize that *DANCR* would be a good candidate to elucidate its role in LSCs.

Long Term Culture - Initiating Cell (LTC-IC) Assay

Murine fibroblasts were cultured to confluency of 80% before the cells were irradiated (80 Gy). CD34 + cells from AML patients were cultured in MyeloCult supplemented with hydrocortisone sodium succinate. Cells were treated with transferrin-NP-siDANCR or transferrin-NP-siSCR containing 300nM of siRNA for 24h in liquid culture prior to seeding the cells in two different concentrations on the feeding layers. The media for the assay was partially changed every week. After 6-weeks of culture, cells were harvested, and equal numbers of cells were seeded in Methocult where colonies were counted after 14 days and the frequency of stem cells was calculated according to the manufacturer's recommendation (Figure 2). We confirmed that *DANCR* expression was significantly higher in the LSC enriched population. These results suggest that a high *DANCR* expression can be used to identifying the LSC-enriched population within an individual patient.

Real Time PCR Expression Measurement

DANCR expression in AML blasts was determined using qRT-PCR in AML patients compared to healthy donor BM. *DANCR* expression was variable between patients, however it was overall elevated significantly compared to healthy controls (Figure 3; $p<0.01$).

***DANCR* Knockdown with Nanoparticle in Colony Forming Unit (CFU) Assay**

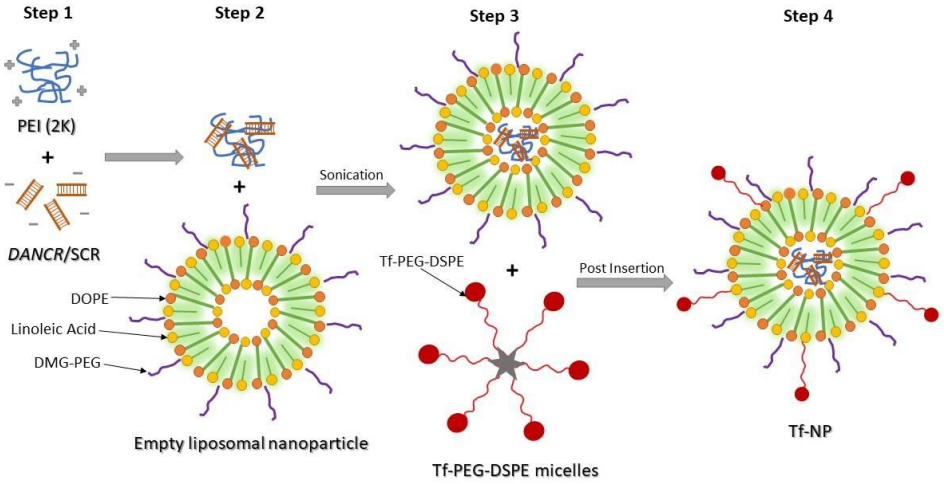


Image 1: Small interfering RNA (siRNA) against human DANCR in Nanoparticle. Briefly, positively charged polyethylenimine was used to capture negatively charged siRNAs, the complex was then loaded into pre-made NPs, which consist of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dimyristoyl-sn-glycerol, methoxypolyethylene glycol (DMG-PEG) and linoleic acid. Transferrin or anti-CD45.2 antibody conjugated with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000] (DSPEPEG2000 maleimide) was then inserted in the NP.

CFU assays were performed by first treating primary CD34+ AML patients' cells with transferrin-NP-siDANCR or transferrin-NP-siSCR containing 300 nM siRNA (Figure 4). After 24 hours of treatment, 2,000-50,000 cells/dish allowed to proliferate for 14 days and then scored for the number of colonies. Cells from primary colonies were then isolated for colony re-plating assays. MethoCult was dissolved in warm RPMI medium containing 20% FBS. Cells were spun, washed with RPMI medium, and counted. Cells were then seeded (10,000-50,000 cells/dish) onto at least duplicate plates without further treatment. Cells were cultured for additional 14 days and then scored.

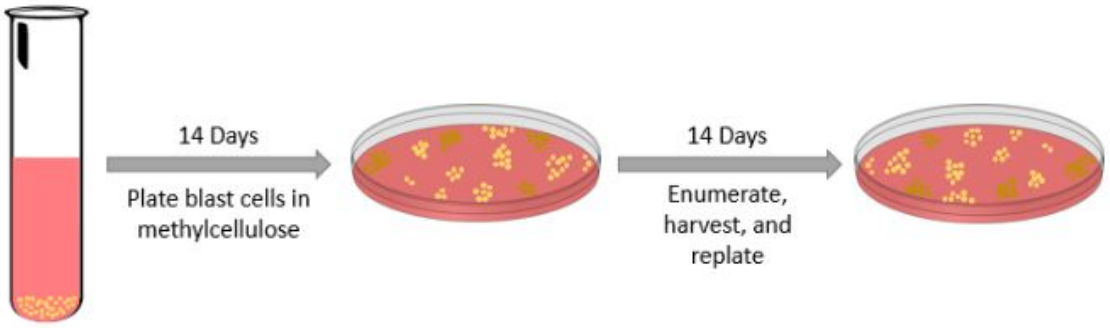


Image 2: Blast cells were harvested after nanoparticle knockdown. Cells were plated and allowed to proliferate before they were recollected and replated without additional treatment.

Using this nanoparticle *DANCR* knockdown method in primary AML blasts, we examined the impact of *DANCR* in LSC self-renewal using colony forming unit (CFU) replating assays. We found significantly fewer colonies in the secondary plating compared to scramble controls (Figure 5; n=3, p=0.03, average decrease~39%). Since LSCs are generating during secondary replating, this indicated a correlation between *DANCR* and LSC self-renewal capacity.

Celltrace Violet - Quiescence Assessment

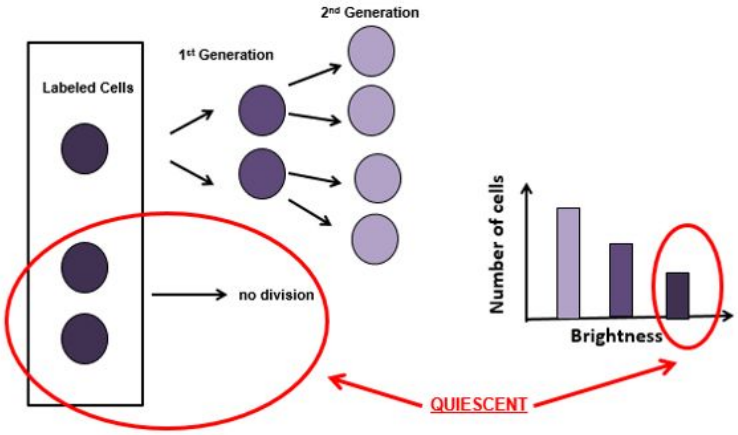


Image 3: Quiescent cells. This image shows that cells that do not divide frequently are considered to be quiescent cells. These are also the most infrequent cell type.

Primary CD34 + AML patients' cells (10 x 10^6) were re-suspend in FBS enriched PBS. A CTV solution was added. Cell suspension was gently vortexed and incubated for 3 minutes in the dark. The cell suspension was directly quenched with cold RPMI medium containing FBS and kept on ice for 5 minutes. Cells were then washed and resuspended in PBS with 2% FBS. Next, cells were sorted using an ARIA II cell sorter for CTV fluorescence. To get a baseline staining, some cells were stained with an anti-CD34 antibody labeled with PEcy7 and 7-AAD viability staining. Next, we demonstrated the differentiation of regularly proliferating cells and quiescent cells. Celltrace violet (CTV) dye was used to assess the effect of *DANCR* knockdown on AML blast quiescence. We found that primary AML blasts treated with Tf-NP-si*DANCR* had a significant decrease in the number of quiescent (CTV max /CD34+) cells ($p<0.05$) compared to Tf-NP-siSCR controls. Figure 6 demonstrates our findings.

Nanoparticle In-vivo Experiments

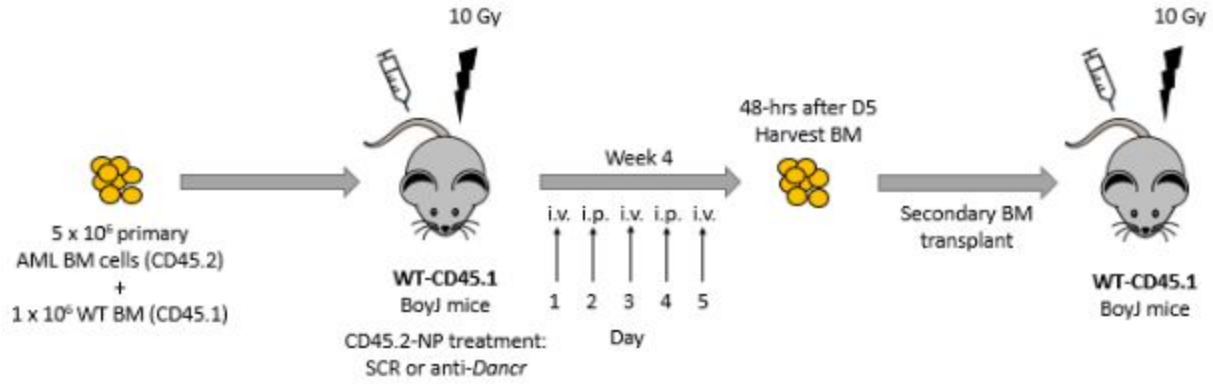


Image 4: Primary AML BM cells are harvested and injected into radiation conditioned mice. These mice were treated with SCR or anti-Dancr before they were sacrificed and their BM was harvested for a secondary transplantation.

First, we transplanted leukemic cells (CD45.2+) into lethally irradiated wild-type (WT)-BoyJ (CD45.1+) mice with whole BM cells from healthy WT-BoyJ donors (CD45.1+). Two weeks after engraftment, mice were treated with NPs conjugated with an anti-CD45.2 antibody to target the leukemic cells.[6, 27] Mice were then treated for five consecutive days via intraperitoneal injection, and were sacrificed 48 hours after their final dosage. Their BM was harvested and sufficient *Dancr* knockdown was confirmed ($p = 0.002$). Next, we determined whether Dancr knock-down affected engraftment in secondary BM transplantation, a key feature of stem cells. Different concentrations (2×10^6, 1×10^6, 5×10^5 of viable cells/mouse) of leukemic donor cells from mice treated with anti-CD45.2-NP-siDancr or anti-CD45.2-NP-siSCR were transplanted into lethally irradiated BoyJ recipients ($n = 5$ per concentration group). Two weeks after transplantation, we found significant decreases in engraftment in mice transplanted with cells from anti-CD45.2-NP-siDancr-treated mice at the two lower cell concentrations (5×10^5: $p = 0.005$; 1×10^6: $p = 0.037$), however no significant decrease was found at the highest concentration (2×10^6: $p = 0.50$) compared with mice transplanted with cells from anti-CD45.2-NP-siSCR control-treated mice (Figure 6). We continued to observe these mice which revealed differences in overall survival, with mice transplanted with cells from anti-CD45.2-NP-siDancr primary donors having significantly longer survival than mice transplanted with cells from the anti-CD45.2-NP-siSCR-treated primary donors. This was found for all concentrations of leukemic donor cells, namely 2×10^6 (median, 62 vs 41 days, $p < 0.001$), 1×10^6 (median, 76 vs 63 days, $p < 0.001$), and 5×10^5 (Figure 8; median, 78 vs 69 days, $p < 0.001$). We calculated the LSC frequency

which showed that Dancr knock-down significantly decreased the number of LSCs per total number of cells ($p < 0.001$).

Section 4: Discussion

Prognosis for AML patients is still poor, and failure to therapies and relapse after achievement of a complete remission present the major clinical challenges.[1,2,7] To improve patients' outcome, attending to LSCs is necessary to eradicate the disease.[13] Thus, a better understanding of LSC biology is necessary to develop novel therapies to target and eliminate the LSCs. We previously showed that LSCs have a distinct microRNA (miR) expression profile.[3,4,6,25] However, the role and significance of lncRNAs in LSCs is not fully understood.

Using the CE-GES score published by Eppert et al.[4] which is based on expression of 44 protein coding genes known to be deregulated in LSCs, we calculated the CE score in 375 younger and 76 older adult CN-AML patients. CE-GES is driven by stem cell biology comparing gene expression signatures associated with LSCs and HSCs. Our analysis using generated a signature consisting of 111 lncRNAs that correlated with CE-GES^{High} in two different data sets of younger and older CN-AML patients.

We further validated the significance of the CE-GES-associated lncRNA signature by demonstrating that one lncRNA, *DANCR*, also plays a functional role in LSCs. We chose *DANCR* to further analyze because it has been shown to be involved in regulating the WNT pathway, which is critical for stem cell functions.[19] It has been shown to be a key regulator in the development stem-like HCC. We identified similar mechanistic results of *DANCR* in AML showing that a knockdown of *DANCR* led to a decrease in the WNT pathway indicating that the mechanism of action of *DANCR* in AML might be similar to that of HCC. It is highly conserved between mouse and

human.[23,24] Importantly, *DANCR* is an intergenic lncRNA, which avoids the possible confounding variable of being co-regulated with its host gene.

Although further analyses are necessary to fully dissect the mechanistic role of *DANCR* in AML cells, especially in LSCs, we have demonstrated for the first time the functional role of *DANCR* in LSCs utilizing cells from AML patients and anti-*DANCR* siRNA loaded transferrin- or antibody-conjugated NPs.[26,27] Our previous studies showed the efficiency of the NP to deliver antagomiRs to LSCs in AML patient-derived samples in xenotransplantation models.[6,26,27] This approach opens the possibility of the first lncRNA-based therapeutic approach for targeting the highly relevant LSC subpopulation.[6,26,27] Our data show that the transferrin/antibody-conjugated NPs result in sufficient knockdown of *DANCR* after only a few doses and without any observable toxicity.

Furthermore, we demonstrate that *DANCR* knockdown had an impact on basic stem-cell features such as self-renewal and quiescence. We translated these data *in vivo* and found that in secondary transplants there was a delay of disease development and prolonged survival of leukemic mice. However, due to the experimental limitations as a result of not being able to isolate an immunophenotypically distinct LSC population, we cannot exclude the possibility that *DANCR* may be involved in other cell processes such as proliferation, survival, or metabolism, among others.

Section 5: Conclusion

In conclusion, we identified an LSC-specific lncRNA signature in AML consisting of 111 lncRNAs. We chose to functionally validate one of these lncRNAs and demonstrate that this lncRNA, *DANCR*, regulates LSC self-renewal and quiescence. Our data conclude that *DANCR* was not only significantly higher expressed in AML cells compared to healthy BM cells but also higher expressed in the LSC enriched compartment within all analyzed patients. To determine *DANCR* function in LSCs, we used a transferrin-conjugated anionic lipopolyplex nanoparticle-based knockdown of *DANCR* to assess its on LSCs. We found *DANCR* regulated LSC self-renewal capacity using CFU replating assays. Furthermore, we found *DANCR* also regulates cellular quiescence using membrane labeling retention assays (CTV). Using nanoparticles containing siRNA-anti-*DANCR*, we demonstrated that *DANCR* can be targeted in vivo and represents a novel target in patients with AML.

These data provide strong evidence that our LSC lncRNA signature may comprise functionally relevant lncRNAs regulating LSC biology and therefore may represent a list of novel LSC-specific therapeutic targets in AML.

Section 6: Impact Innovation

A better understanding of AML pathogenesis and biology is crucial for the hopes of improving patient outcomes. Distinguishing the role of lncRNA in LSCs has the potential to identify novel targets for treating AML patient. For the first time, we have identified a role for the lncRNA, *DANCR*, to regulate LSCs in AML. We have dissected *DANCR*'s contribution to leukemogenesis by investigating the effects of *DANCR* knockdown on cell self-renewal and partial maturation abilities. Our goal is to one day use this knowledge to develop personalized novel therapies for each AML patient. Understanding the biology and mechanisms regulating LSCs will open the doors to personalized treatment. This would not only allow for better responses to initial therapies but could also prevent minimal residual disease and relapse.

Section 7: Supplemental Material

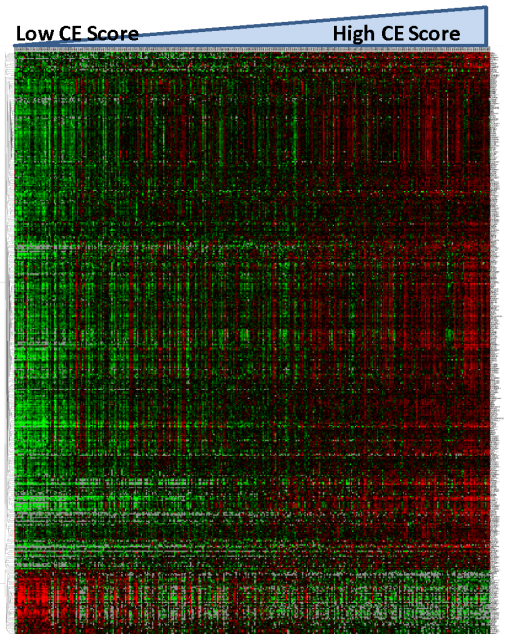


Figure 1 - (A) Heat map of 365 younger (aged <60 years) adult patients with cytogenetically normal AML (CN-AML). Each column represents a single patient. Patients were classified according to their core enriched (CE) gene expression signature (GES). Patients on the left side had a lower CE-GES score while patients on the right side were CE^{High}. Each row indicates a lncRNA that was significantly deregulated between CE^{Low} and CE^{High} patients; these lncRNAs were cross validated in a set of 76 older CN-AML patients. Expression values of the lncRNAs are represented by color, with red indicating higher expression and green indicating lower expression.

	concentration	CD34+ anti SCR Frequency 1 LSC per n cells	CD34+ anti *DANCR* Frequency 1 LSC per n cells	*P*
Patient 302	100 nM	1.2*10^2	2.6*10^2	.13
Patient 302	300 nM	1.2*10^2	5.8*10^2	.001
Patient 888	100 nM	6.0*10^1	1.5*10^2	.0008
Patient 888	300 nM	4.6*10^1	2.8*10^2	.003

Figure 2 – LTC-IC results of 3 AML patient. A knock-down of *DANCR* leads to a significant decrease in LSC frequency

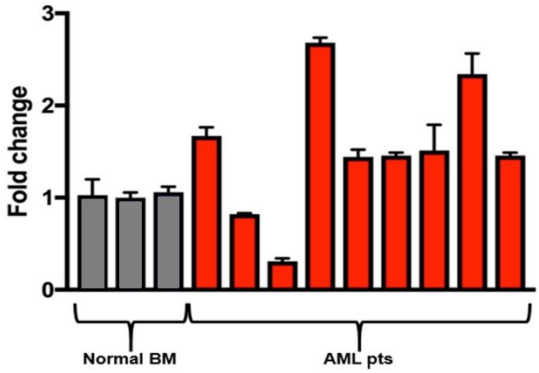


Figure 3 - qRT-PCR of healthy bone marrow (n=3) and AML patients marrow (n=9). Although *DANCR* expression varied, it was significantly higher expressed in several patients with AML (P<0.05)

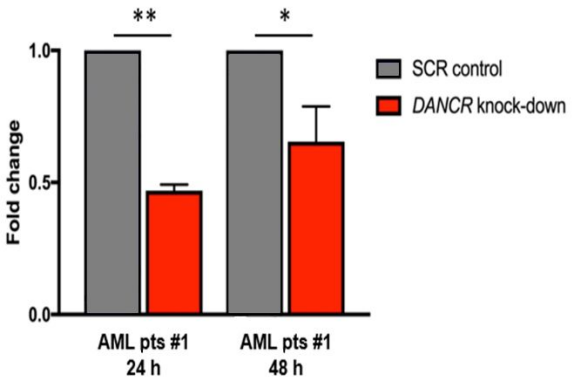


Figure 4 - Target and knock-down of *DANCR* using loaded nanoparticle (NP-anti-*DANCR*) compared to a scramble control (NP-SCR). Transferrin was added to target LSCs. Data of CD34+ enriched AML patient cells (*P<0.05, **P<0.01)

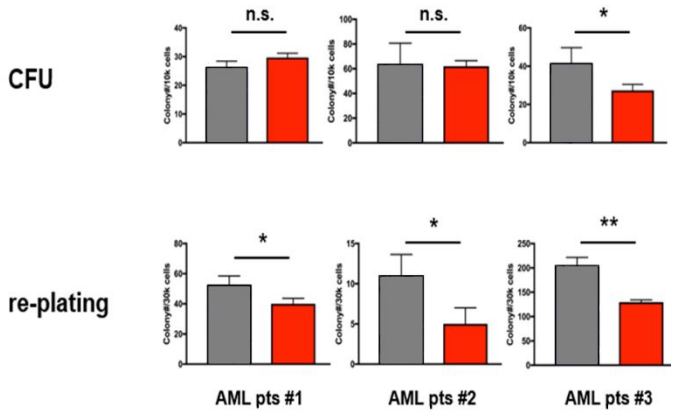


Figure 5 - Colony forming units (CFUs) were performed by using NP-anti-*DANCR* or scramble control (NP-SCR) treated cells plated in methylcellulose media. Cells were then replated to evaluate self-renewal capacity, a characteristic of LSC (*P<0.05, **P<0.01)

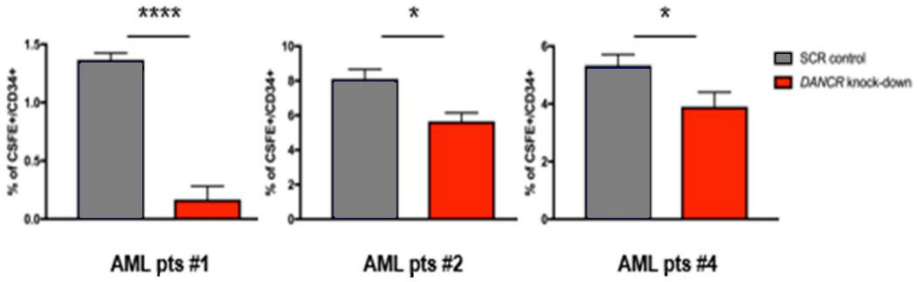


Figure 6 - Quiescent cells are cells that rarely divide; a characteristic of LSCs Cell trace violet (CTV) proliferation assays were performed on AML blasts treated with NP-anti-*DANCR* and NP-SCR with CTV dye and were cultured for three days. Quiescent CTVmax/CD34+ cells (*P<0.05, ****P<0.0001)

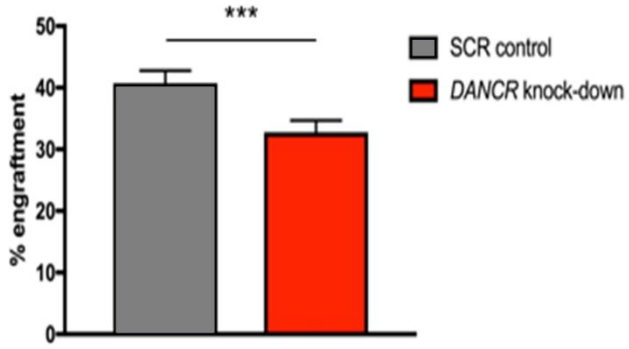


Figure 7 - Three dose concentrations of harvested cells were administered to BoyJ mouse recipients (n=5 per group). Engraftment of treated mice were compared (***P<0.001).

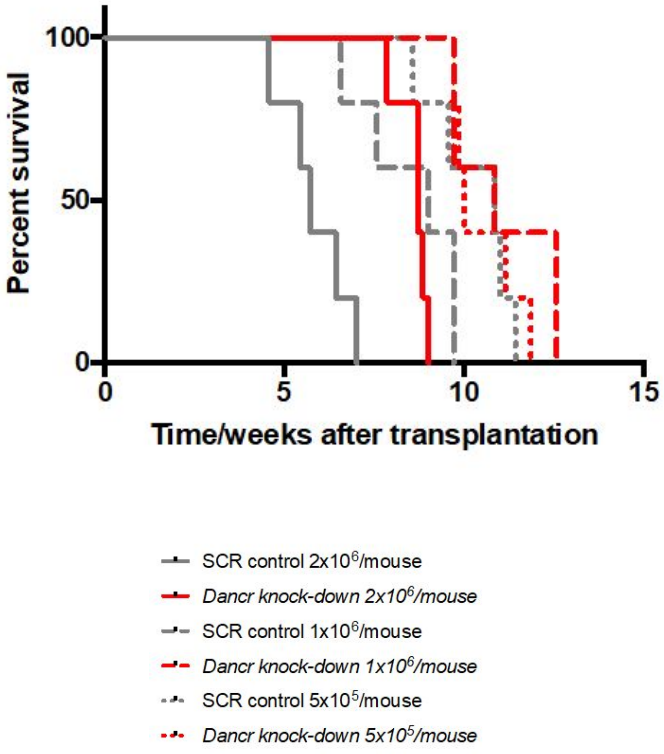


Figure 8 - Three dose concentrations of harvested cells were administered to BoyJ mouse recipients (n=5 per group). Engraftment of treated mice were compared (***P<0.001).

Bibliography

1 Dohner H, et al. Diagnosis and management of acute myeloid leukemia in adults: Recommendation from an international expert panel, on behalf of the European Leukemia Net. *Blood*. 2010.

2 Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373(12):1136-1152.

3 Metzeler KH, et al. A stem cell-like gene expression signature associates with inferior outcomes and a distinct microRNA expression profile in adults with primary cytogenetically normal acute myeloid leukemia. *Leukemia*. 2013;27(10):2023-2031.

4 Eppert K, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. *Nat Med*. 2011;17(9):1086-1093.

5 Dick JE. Stem cell concepts renew cancer research. *Blood*. 2008;112(13):4793-4807.

6 Dorrance AM, et al. Targeting leukemia stem cells in vivo with antagomiR-126 nanoparticles in acute myeloid leukemia. *Leukemia.* 2015;29(11):2143-2153.

7 Ng SW, et al. A 17-gene stemness score for rapid determination of risk in acute leukemia. *Nature*. 2016;540(7633):433-437.

8 Sagar J, et al. Role of stem cells in cancer therapy and cancer stem cells: a review. *Cancer Cell Int.* 2007;7:9.

9 Sarry J-E et al. Human acute myelogenous leukemia stem cells are rare and heterogenous when assayed in NOD/SCID/IL2Ryc-deficient mice. *J Clin Invest.* 2011;121:384-395.

10 Heidel FH, Mar BG, Armstrong SA. Self-renewal related signaling in myeloid leukemia stem cells. Int J Hematol. 2011;94:109-117.

11 Guan Y, Gerhard B, Hogge DE. Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). Blood. 2003;101(8):3142-3149.

12 Jentzsch M, et al. Prognostic impact of the CD34+/CD38- cell burden in patients with acute myeloid leukemia receiving allogeneic stem cell transplantation. Am J Hematol. 2017;92(4):388-396.

13 Misaghian N, et al. Targeting the leukemic stem cell: the Holy Grail of leukemia therapy. Leukemia. 2009;23(1):25-42.

14 Guzman ML, et al. An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. Blood. 2007;110:4427-4435.

15 Garzon R, et al. Expression and prognostic impact of lncRNAs in acute myeloid leukemia. Proc Natl Acad Sci U S A. 2014;111(52):18679-18684.

16 Fang Y, Fullwood MJ Roles, Functions, and Mechanisms of Long Non-coding RNAs in Cancer. *Genomics Proteomics Bioinformatics* (2016), http://dx.doi.org/10.1016/j.gpb.2015.09.006

17 Yuan SX, Wang J, Yang F, Tao QF, Zhang J, Wang LL, Yang Y, Liu H, Wang ZG, Xu QG, Fan J, Liu L, Sun SH, Zhou WP. Long noncoding RNA *DANCR* increases stemness features of hepatocellular carcinoma by derepression of CTNNB1. Hepatology. 2016;63(2):499-511.

18 Luo M, et al. Long non-coding RNAs control hematopoietic stem cell function. Cell Stem Cell. 2015;16(4):426-438.

19 Harrow J, et al. GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res. 2012;22(9):1760-1774.

20 Kretz M, et al. Suppression of progenitor differentiation requires the long noncoding RNA *DANCR*. Genes Dev. 2012;26(4):338-343.

21 Lu Q-C, Rui Z-H, Guo Z-L, Xie W, Shan S, Ren T. LncRNA-*DANCR* contributes to lung adenocarcinoma progression by sponging miR-496 to modulate mTOR expression. J Cell Mol Med. 2018;22(3):1527-1537.

22 Jiang N, et al. lncRNA *DANCR* promotes tumor progression and cancer stemness features in osteosarcoma by upregulating AXL via miR-33a-5p inhibition. Cancer Lett. 2017;405:46-55.

23 Breschi A, Gingeras TR, Guigó R. Comparative transcriptomics in human and mouse. Nat Rev Genet. 2017;18(7):425-440.

24 Zhou D, et al. Integrative genomic analyses reveal clinically relevant long non-coding RNA in human cancer. Nat Struct Mol Biol. 2013;20(7):908-913.

25 Papaioannou D, et al. Prognostic and biological significance of the proangiogenic factor EGFL7 in acute myeloid leukemia. Proc Natl Acad Sci U S A. 2017;114(23):E4641-E4647.

26 Raffel S, et al. BCAT1 restricts αKG levels in AML stem cells leading to IDH^{mut}-like DNA hypermethylation. Nature. 2017;551(7680):384-388.

27 Huang X, et al. Targeted delivery of microRNA-29b by transferrin-conjugated anionic lipopolyplex nanoparticles: a novel therapeutic strategy in acute myeloid leukemia. Clin Cancer Res. 2013;19(9):2355-2367.